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QUANTITATIVE IRRADIATION EXPERIMENTS WITH NEUROSPORA CRASSA. II. ULTRAVIOLET IRRADIATION

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QUANTITATIVE IRRADIATION EXPERIMENTS WITH NEUROSPORA CRASSA. II. ULTRAVIOLET IRRADIATION [1]

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IT HAS been found that exposure to ultraviolet irradiation at sublethal doses will induce changes of a mutation-like character in *Trichophyton mentagrophytes* (Emmons and Hollaender, 1939; Hollaender and Emmons, 1941). These experiments were repeated with *Neurospora,* a fungus in which such changes could be subjected to genetical analysis. A similar study was conducted on the effects of X-rays on this organism. The results of X-ray treatment on mutation production are given in a separate paper (Sansome, Demerec, and Hollaender, 1945). In this report we will describe the effects of ultraviolet irradiation and discuss them in relation to the X-ray results.[2] Every effort was made to keep the conditions and techniques in the X-ray and the ultraviolet experiments as constant as possible, and the discussion of materials and all genetic and nonphysical methods given in the first paper are applicable here.

ULTRAVIOLET IRRADIATION TECHNIQUE.—Microconidia were washed off with physiological salt solution, shaken thoroughly, filtered through absorbent cotton, and centrifuged in an angle centrifuge at about 4000 rotations per minute for 30 minutes. The precipitated spores were resuspended in physiological salt solution and shaken to break up any possible clumps. The approximate number of spores was determined in a Petroff-Hausser bacterial counting chamber, and usually was about 100,000 to 1,000,000 per cc.

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Appreciation is expressed to Mrs. M. B. Houlahan, who was connected with the earlier part of the study.

[2] The data on mutation, wavelength and dosage were obtained at the National Institute of Health. The second author accepts responsibility for the interpretation of results from the genetical aspect.

The radiation apparatus used in this study was substantially the same as that used in previous studies (Hollaender and Claus, 1936; Hollaender and Emmons, 1939; Emmons and Hollaender, 1939). The radiation of a medium-pressure water-cooled quartz capillary mercury vapor lamp of the Daniels-Heidt type was concentrated on the entrance slit of a quartz monochromator. The emerging monochromatic beam was concentrated on a standardized thermopile for the determination of the energy or the face of the exposure cell. All lenses, prisms, and windows were made of quartz with good transmission down to 2000 A.

The material was exposed to the radiation in a cell which permitted the removal of 1/10-cc. samples during irradiation without interrupting the exposure. The material was stirred very thoroughly, to make certain that each spore received an equivalent amount of energy.

There are two ways in which to get a true picture of the amount of radiation each spore receives; one is to have a dense suspension which will absorb all the energy entering the exposure cell. The other method is to have a very dilute suspension in which practically all the energy entering the exposure cell is transmitted; i.e., the number of spores is so low that the amount of energy absorbed cannot be measured. In both techniques since the beam of ultraviolet does not cover the entire cell it is imperative that the material be stirred constantly, because it is often difficult to get a suspension of spores of high concentration. For this reason the method of irradiating very dilute suspensions was used. The amount of energy received by each cc. of spore suspension was determined for the incident energy at

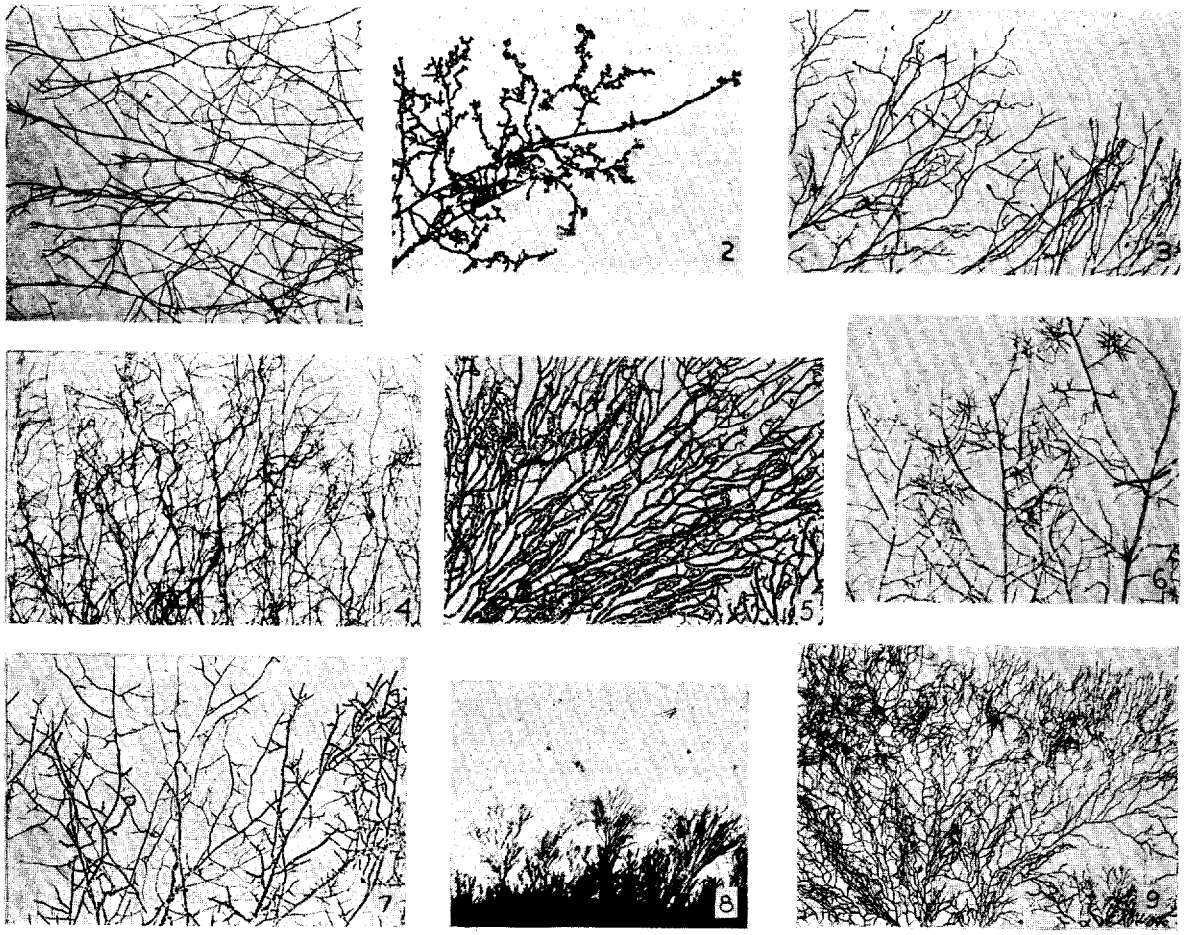

Fig. 1–9.—Fig. 1. Typical mycelium of *Neurospora crassa* (fluffy) coming from culture used as control.—Fig. 2 to 9. Different mutant types showing variation in branching and growth. All figures enlarged from original. The cultures were grown along a bare glass surface and photographed after 24 hour growth with exception of cultures of figures 2 and 9 which were photographed after 48 hours.

the exposure cell divided by the number of cc. irradiated. A typical calculation follows:

T = time of exposure in seconds.
I = energy per centimeter deflection of galvanometer in ergs per second.
d = centimeter deflection of galvanometer.
K = cubic centimeters per exposure cell.

$$\frac{d \times I \times T}{K} = \text{ergs per cc.}$$

The quantity that was removed for each exposure (1/10 cc.) was taken into account. A control sample was taken before each experiment was started. The control and experimental samples were diluted in physiological saline and plated out in different dilutions. The spores were isolated on germination and transferred to small tubes.

A method which was found very useful with other fungi (*Trichophyton mentagrophytes,* Hollaender and Emmons, 1939; *Aspergillus terreus,* Hollaender, Raper, and Coghill, 1945), namely, the determination of the survival rate after exposure to ultraviolet, was not readily applicable to this study. *Neurospora crassa* has a spreading growth habit which makes it very difficult to determine the numbers that germinate on a particular plate. This difficulty is increased by the fact that the spores do not all germinate at the same time. Ultraviolet-irradiated spores show an even greater variability in time of germination than untreated spores. In general ultraviolet-irradiated spores grow more slowly at first and the extent of this slowing up of the growth rate is roughly a function of the energy which the spores had received.

Types of mutants recorded.—As in the X-ray experiments, cultures that were visibly different from normal were recorded as mutants. Certain of the cultures appeared mutant at first and later reverted to normal. When the reversion occurred in the first week, the cultures were classified as normal, but when it occurred later the cultures were recorded as mutants. Out of 303 mutants, 80 were colonial. The majority showed less than normal vigor. The grossly different appearance of the mu-

tants was usually correlated with a difference in branching habit as seen under the microscope. Figures 1 to 9 illustrate the branching habits of a few of the mutants found. A class of mutants that showed very reduced growth and usually either reverted to normal or died out on successive transfers was found. These mutants probably belong to the type called "degenerate phenotypes" by Lindegren and Lindegren (1941), and their occurrence seems

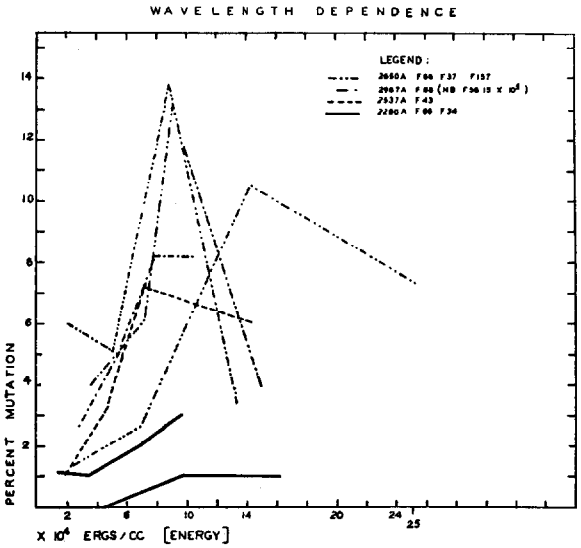

Fig. 10. Wavelength dependence of mutation production at 4 wavelengths. The energy values which will have to be multiplied by 10^6 for 2650, 2537, and 2280 should be multiplied by 10^5 for 2967.

to be a characteristic of ultraviolet irradiation. Out of 303 mutants, 46 which died out may be presumed to belong to this class. Some of the 68 reversions that occurred probably belong to this class also; but, as will be discussed later, reversion may be due to several causes.

WAVELENGTH DEPENDENCE.—A set of seven wavelengths, namely, 2280, 2380, 2480, 2537, 2650, 2805, and 2967 A were compared as to their effectiveness in mutation production. The work of Hollaender and Emmons (1941) demonstrates that the energy necessary for mutation production is lowest at 2650 A with a second minimum at 2280 and is highest at 2967. Because of these findings the regions of the ultraviolet spectrum illustrated in figure 10, namely 2280, 2650, and 2967, were emphasized in this study. For equivalent energy value, the killing rate was very high and the mutation rate

TABLE 1. *Frequency of visible mutations produced by different wavelengths at various dosages.*

Wavelength	Experiment	Energy in ergs/cc. × 10^4	Number tested	Per cent mutation
		0	50	0
		4.44	100	0
2280 A	F 34	9.82	100	1
		16.18	100	1
		0	60	0
		1.36	191	1.1
	F 66	3.40	194	1
		6.81	205	2
		9.57	197	3
		0	53	0
		1.94	98	1
2537 A	F 43	4.38	97	3.1
		7.14	97	7.2
		14.32	83	6.0

TABLE 1. *Concluded.*

Wavelength	Experiment number	Energy in ergs/cc. × 10^4	Number tested	Per cent mutation
		0	59	0
		28.4	81	2.6
2967 A	F 58	53.0	96	5.2
		78.1	72	8.2
		103.5	98	8.2
2650 A		0	51	0
		3.67	100	4
	F 37	7.12	100	6
		11.08	100	13
		14.99	100	4
		0	86	0
		3.79	106	.9
	F 52	7.17	100	1
		14.87	75	4
		19.97	96	7.2
		0	82	2
		7.65	102	5.9
	F 59	13.20	90	10
		19.30	100	5
		28.80	115	3.4
		0	64	0
		2.14	199	6
	F 68	4.96	198	5.1
		8.68	196	13.9
		13.58	183	3.3
		0	118	0
		2.31	240	1.7
	F 157	6.98	230	2.6
		14.27	229	10.5
		25.32	234	7.3
		0	108	0
		3.19	100	1
	F 17	7.13	100	0
		11.00	93	3.2
		16.52	91	1.1
		21.74	98	1
		40.64	99	4.0
		0	50	0
		6.80	311	1
	F 22	19.68	324	.3
		44.88	286	1.4
		84.94	292	3.8
		0	115	1
		5.03	111	1.8
	F 49	8.72	116	1.8
		12.92	83	7.2
		20.47	107	4.6

TABLE 2. *Effects on survived of seven wavelengths at different energies.*

Wavelength	Experiment number	Energy—ergs/cc. × 10^4	Number[a] spores per cc.	Per cent survival
2280 A	F 34	0	325 × 10^2	100
		4.4	163 × 10^2	50
		9.8	71 × 10^2	21.9
		16.7	15.5 × 10^2	4.7
	F 42	0	84 × 10^3	100
		3.8	127 × 10^2	15.2
		6.5	42.3 × 10^2	5.1
		9.1	73 × 10	.8
		15.1	43 × 10	.5
2380 A	F 45	0	72.6 × 10^3	100
		2.4	52.5 × 10^3	72.6
		7.2	120 × 10^2	22.6
		16.6	59.6 × 10^2	8.1
		33.7	109 × 10	1.5
2480 A	F 46	0	104.5 × 10^3	100
		4.3	156.5 × 10^2	15
		7.9	25 × 10^2	2.5
		15.9	91 × 10	.8
		27.8	48.6 × 10	.4
2537 A	F 43	0	63 × 10^3	100
		1.9	72.6 × 10^3	+
		4.3	95.5 × 10^2	15.3
		7.1	18 × 10^2	2.3
		14.3	46 × 10	.7
2650 A	F 23	0	23.3 × 10^3	100
		4.2	15 × 10^3	35
		7.7	17 × 10^2	10
		16.2	13 × 10	poor
	F 27	0	88.5 × 10^2	100
		2.9	28.5 × 10^2	29.9
		6.6	40.5 × 10	4.7
		13.4	37.5 × 10	4.4
	F 37	0	97.5 × 10^3	100
		3.3	78 × 10^3	67
		6.8	118 × 10^2	14
		10.7	84.3 × 10	8.3
		14.7	84.5 × 10	.9
	F 41	0	179 × 10^2	100
		2.5	60 × 10^2	33.5
		4.9	61 × 10^2	3.4
		8.3	36 × 10	2.0
		13.6	23 × 10	1.3
2805 A	F 44	0	80.5 × 10^3	100
		5	50.2 × 10^3	62.4
		8.3	12.6 × 10^2	15.6
		14.5	36 × 10	4.5
		23.2	97 ?	1.2
2967 A	F 40	0	39 × 10^3	100
		36	202 × 10^2	52
		56.7	112 × 10^2	28.8
		81.2	29 × 10^2	7.4
		137.8	208 × 10	5.3

[a] The first figure in column 4 gives the average of the counts on two to four plates multiplied by the dilution factor which then gives the number of colony-forming organisms per cc.

low at a wavelength of 2280; and at 2967 the killing and mutation rates were both low. It required a tenfold increase in energy at 2967 to cause killing and mutation rates of the order of magnitude given by 2650 A (tables 1 and 2).

MUTATION FREQUENCY AND DOSAGE.—Because there appears to be a certain variability in the response to ultraviolet treatment of spore suspensions coming from different cultures, the experiments cannot be averaged. Table 1 includes the results of eight experiments at a wavelength of 2650, the wavelength that was found to be most efficient in producing mutations. These experiments were selected from a number of others because they happened to have fairly high numbers of spores at each dosage. They are representative of the results obtained in other experiments. In the case of F37, F17, F22, and F41, fluffy + strains were used; and in the remaining experiments, fluffy — strains. It will be noticed that in five experiments the mutation rate increases with energy up to a certain level and then decreases with a further increase in energy.[3] The peak of the curve occurs at approximately the same energy level, but its height varies from experiment to experiment. Experiment F32 does not show this behavior, nor do experiments F17 and F22. In the last two experiments the mutation rates are very low, and therefore the results are not very instructive. Moreover, in the case of F22 most of the runs involved very high energy values, at which values the maximum mutation rate would have been passed according to the results of the other experiments. The same strain was used in experiments F17 and F22, and it is possible that this was a strain with a low mutation rate. Such strains were also found in *Trichophyton* (Emmons and Hollaender, 1939).

THE FREQUENCY DOSAGE CURVE.—One explanation of a curve of this type, which is unique in irradiation experiments, is that the spore suspension is heterogeneous in respect to the amount of radiation absorbed by each spore or in the response to irradiation. The conditions of irradiation seem to preclude the idea of differential exposure of the individual spores. The possibility that the spores themselves are different in their response remains to be considered.

In measuring the mutation rate we are measuring the effect of the ultraviolet irradiation on the nucleus. However, there are two main ways in which the spores may be heterogeneous in respect to their mutational response to ultraviolet irradiation. The cell wall and the cytoplasm surrounding the nucleus may differ in their capacity to transmit the ultraviolet radiation at different stages, or the nucleus may give a different response at different stages of its cycle. One or both of these factors may be operative. Several experiments were made in the

[3] Similar curves have been found in *Penicillium notatum* and *Aspergillus terreus* (Hollaender and Zimmer, 1945; Hollaender, Raper and Coghill, 1945).

TABLE 3. *Frequency of mutations in young and old irradiated spores.*

Experiment number	Young spores			Old spores		
	Energy	Number tested	Per cent mutation	Energy	Number tested	Per cent mutation
	0	52	0	0	53	0
	7.30	154	4.5	6.46	148	3.3
F 13	20.22	155	11.7	20.18	154	2.6
	43.42	145	10.6	45.37	129	1.6
	0	50	0	0	48	1
	4.61	24	1	5.75	86	1
F 113	14.67	88	5	15.45	89	3
	31.02	93	4	25.09	104	1
	0	50	0	0	47	0
	8.80	106	1.9	8.16	98	4.2
F 121	24.28	100	2.0	25.36	98	9.2
	49.74	106	1.9	48.26	108	4.6

hope of obtaining evidence regarding the heterogeneity of the treated spore samples.

IRRADIATION OF OLD AND YOUNG SPORES.—It was thought that if variations occurred, either in the capacity to absorb ultraviolet radiation or in the power of the nucleus to respond to the treatment, samples of predominantly young spores might differ from those of predominantly older spores in their mutation rate after ultraviolet irradiation. Consequently, comparable cultures were wetted down, and microspore suspensions prepared and irradiated after 24 hours and after 48 hours. Twenty-four hours after wetting, the number of microconidia was relatively low, and spores had to be taken from 15–20 cultures in order to obtain suspensions of a desirable density. The results of three experiments involving young and old conidia are given in table 3. These results are insufficient; but it will be seen that, whereas in experiments F113 and F13 the mutation rates of the young conidia were higher, in experiment F121 the reverse occurred. Thus, while the data do not exclude the possibility of a different mutational reaction of young and old conidia to ultraviolet irradiation, they afford no evidence of any consistent effect of that kind.

ANALYSIS OF NORMAL PHENOTYPES IN RELATION TO CHROMATID EFFECTS.—Stadler and his associates found that the endosperms from crosses involving ultraviolet-treated pollen of *Zea Mays* showed a great number of fractional deficiencies (Stadler and Uber, 1942). They suggest that these deficiencies are explicable if only one chromatid derived from a treated chromosome is affected, the other being normal. It is desirable to consider the results of such chromatid effects in *Neurospora*. If, as an effect of ultraviolet irradiation, one of the two nuclei resulting from the first nuclear division after treatment should be normal, and the other mutant, the normal nucleus might be expected to predominate over the mutant so that the resulting culture would appear normal. In *Neurospora*, therefore, chromatid effects would be masked, and only chromosome effects would be visible. The following simple hypothesis to explain the rise and subsequent fall in the mutation rate was considered. It was assumed that chromatid effects in general are not visible, only chromosome effects appearing as visible mutants. It was further assumed that the type of effect, whether chromatid or chromosome, is conditioned largely by the nuclear stage of the microconidium undergoing treatment, only microconidia at a certain stage being capable of full chromosome effects. On these assumptions, the mutation rate would rise with dosage until every conidium had received approximately one effect. At this point those microconidia that were capable only of a chromatid response would appear phenotypically normal, since the mutant would be covered by the normal allele. Of the microconidia that gave a chromosome response to the ultraviolet treatment, those undergoing lethal mutations would be eliminated and the remainder would appear as visible mutants. An increase in dosage leading to coincident mutations would result in a reduction in the number of visible mutants in the class of microconidia giving chromosome effects, because of the coincidence of lethal and visible mutations. The extent of the reduction of mutation rate would depend upon the relative proportions of visible and lethal mutations. In the case of microconidia giving chromatid effects only, the occurrence of two mutations in one nucleus would be followed by the separation of the two mutants into one nucleus in half the cases, when the remaining normal nucleus would be expected to predominate. In the other half of the cases the two mutations would pass to different nuclei and might be expected to produce a balanced heterokaryon. In experimentally made heterokaryons between pairs of visible mutants, it was found that nine out of fourteen combinations appeared normal. Hence a high proportion of balanced heterokaryons might be expected to appear normal eventually, although they might not achieve a balance at first, in which case they would first appear mutant and later would appear normal.

It was thought, therefore, that the microconidia giving only chromatid responses to ultraviolet irradiation would continue to appear normal even when two or more mutations were present, owing to the balanced heterokaryon phenomenon, whereas the number of visible mutants from the microconidia giving chromosome effects would decrease because of the coincidence of lethal and visible mutations in a certain proportion of cases. This would account very well for the dosage-mutation curve actually found. On this hypothesis, a fair proportion of phenotypically normal cultures from heavily treated microconidia would be expected to be balanced heterokaryons. Accordingly, a microconidial analysis of a number of these normals was made. Subcultures about one week old were wetted down, and the microconidia so obtained were plated out and later isolated into tubes. Out of twenty cultures from a spore sample given a dosage of 17.9×10^4 ergs per cubic centimeter at 2650 (experiment F12, run 5), one was a balanced heterokaryon consisting of two semi-lethal mutants, four gave one mutant from approximately 30 isolated microconidia, and fifteen gave only standard fluffy cultures. Out of 16 cultures given a dosage of 21.7×10^4 ergs per cc. (experiment F17, run 5), all gave only fluffy cultures; and out of 13 cultures given a dosage of 40.6×10^4 ergs per cc. (experiment F17, run 6), only fluffy cultures were obtained in all cases except one, where one mutant was obtained. Thus, out of 49 cultures tested, only one was a balanced heterokaryon. Five were possibly heterokaryotic for normal and a mutant character; but, since the microconidia came from subcultures taken from old cultures, these mutants may have been due to spontaneous mutation and not to chromatid mutation during irradiation. As will be seen later, some of the reversions were probably balanced heterokaryons; but, since they appeared mutant at first and were so considered when the data for the dosage-mutation tables were collected, they cannot be considered as helping to keep up the proportion of normal phenotypes at high dosages. The results on the microconidial analysis of normal phenotypes recovered from high dosages, therefore, seem to eliminate the hypothesis that the drop in the dosage-mutation curve is due to heterogeneity of microconidia with respect to their capacity to give a chromosome or chromatid response to ultraviolet irradiation.

TABLE 4.

Class	Number of cultures	Types recovered
A	4	Fluffy only
B	4	Dwarf or scant at first; later fluffy
C	5	Mutant only
D	11	Mutant and fluffy
E	11	More than one type of mutant

REVERSIONS AND CHROMATID EFFECTS.—As mentioned before, a number of cultures that were mutant at first later reverted. The general nature of unstable forms is discussed in the X-ray paper. Out of 303 mutants, 68 reverted sooner or later. No correlation could be found between the frequency of reversion and the wavelength or dosage. A microconidial analysis of 35 reverted mutants gave the results summarized in table 4.

The mutants falling into class A may be of three types: (1) Genuine reverse mutations, in which the "fluffy" nuclei have overgrown the original mutant type. In this case it should be possible to recover mutants from crosses involving the original culture before it reverted. (2) A type described by the Lindegrens as "degenerate phenotype," which gives only wild-type and fluffy progeny when crossed with a standard wild-type line. Such types most probably result from an effect of the ultraviolet radiation on the cytoplasm. (3) Normals wrongly classified as mutants.

Class B seems to consist of a special type of mutant which is slow-growing at first but attains normal vigor after growing for a time. Ascospores from mutants of this class appear dwarf or scanty at first and normal later.

In the case of class C, in which only mutants were recovered, three explanations are possible. (1) The phenotypic reversion might be due to a gene mutation to normal fluffy, but the original mutant nuclei might be still so numerous that a small spore sample would detect only mutants. (2) The culture on growing might acquire a cytoplasmic adaptation to the mutant gene which is lost when single conidial cultures are taken. This might well be a more extreme example of the type of phenomenon shown by class B mutants. (3) The third possibility is that the original culture consisted of a balanced heterokaryon between a lethal mutation and a visible mutation. On microconidial analysis, only the visible mutants would be viable and therefore recoverable. In this case we must assume that two chromatid changes occurred at the time of irradiation, one to a visible mutation, the other to a lethal, and that these were distributed to different nuclei at the first nuclear division. The culture appeared mutant at first, but after a favorable ratio had been established between the two types of nuclei it appeared normal.

Class E, in which more than one type of mutant was obtained, evidently consists of balanced heterokaryons involving two visible mutants. Class D consists of heterokaryons between fluffy and a fluffy mutant type. It is difficult to know whether these types were heterokaryotic from the time of irradiation or whether they were entirely mutant at first and became heterokaryotic by mutation. Each particular mutant would have to be investigated carefully, to show which is the more probable explanation.

However, in the case of class E, and probably of class C also, we have fairly clear evidence of a

chromatid rather than a chromosome effect of the irradiation. Since these cases involve coincidental chromatid changes, it would seem that chromatid changes are quite frequent, as might have been expected from Stadler's results on maize. Moreover, it may well be that some of the non-reverted visible mutants are heterokaryons.

Analysis of balanced heterokaryons should afford a method for estimating the relative proportions of visible and lethal mutations. The present data, in which there were 11 balanced heterokaryons involving two visible mutations (class E) and 5 involving possibly one visible and one lethal (class C), might indicate that lethals are less frequent than visibles; but a more extensive analysis is needed to settle this question.

THE OCCURRENCE OF MULTIPLE MUTATIONS.—In view of the possibility that the spore samples were heterogeneous in their response to ultraviolet radiation, it was thought advisable to determine, if possible, the proportion of coincidental mutations among the ultraviolet-induced mutants. Accordingly, ascospores from crosses involving some of the flufly mutants and the wild-type were grown, and the progeny recorded. In crosses involving twenty-eight mutants, whole asci were dissected and the ascospores removed in linear order; in the case of forty other mutants, ascospores from the cross with wild-type were sown at random. The results from the single-ascus dissections were: thirteen cases in which one mutant type was recovered together with fluffy and wild-type; eight cases in which no mutant was recovered, although more than four spores germinated; three cases in which no mutant type was recovered, but only four spores or less germinated; and four cases in which more than one mutant type was recovered. If the eight types in which no mutant was recovered belong to the degenerate-phenotype class described by Lindegren and are not gene mutations, then out of twenty genetic mutants four involve two or more genetic loci. From the forty mutants subjected to random ascospore analysis, more than one mutant type was recovered nineteen times, and only one mutant type twenty-one times. The discrepancy between the results from whole-ascus dissections and those from random ascospore sowing may be partly due to the fact that for some crosses very few asci were dissected, so that a second mutant type might have been present and not recovered. In any case, the numbers are small and the difference is not significant. There is no doubt that the finding of twenty-four mutants with two or more mutational changes out of sixty investigated indicates a very high degree of coincidence of mutations. No correlation could be detected between dosage and the occurrence of coincidental mutations.

STERILITY.—Since visible mutations were found to be correlated with sterility in the case of the X-ray-induced mutants, a number of the ultraviolet-induced mutants were investigated for sterility by the technique used in analyzing the X-ray mutants. Of forty-seven mutants so examined, forty were as fertile as the controls, three were partly sterile, two had empty perithecia, and two were doubtful part-steriles. It is difficult to make a close comparison between the X-ray and ultraviolet results, because the ultraviolet mutants were from samples that had been subjected to different amounts of radiation and that gave different mutation rates. However, in the X-ray cultures, even at the lowest dosage examined, about 50 per cent of the mutants were partly sterile. Thus there is a distinct difference in frequency of sterility in the ultraviolet- and X-ray-induced mutants, although ultraviolet-induced mutants may sometimes be sterile.

DISCUSSION.—In a study of the effects of seven wavelengths between 2200 and 3000 A, three were especially studied—namely, 2650, 2280, and 2967 A. At 2650, nucleic acids have high absorption; and this wavelength is most efficient in producing killing and mutation effects; that is, lower amounts of energy are required to produce a given effect at this wavelength than at the others. At 2280, nucleic acids, proteins, and other constituents of the cell—including the cell wall—absorb the radiation rather intensely, as is shown by photographs taken at this wavelength (Cole, 1941). Therefore it is likely that at 2280 most of the radiation to which the cell is exposed will be absorbed by the cell wall and the cytoplasm. This might be expected to kill the cell rather than produce changes in the nucleus, and this is what actually seems to occur. At 2967, very little measurable absorption is found; but with very high dosages both killing and mutational effects can be obtained.

The typical ultraviolet dosage-mutation curve rises to a maximum, and with a further increase of dosage the percentage of mutations decreases. There is a certain variability in mutation rate among spore samples. A genetical analysis of the mutants obtained shows that the proportion of multiple mutations is high. A possible explanation of the dosage-mutation curve, based on the assumption that only a certain proportion of the spores can give a full chromosome response to ultraviolet irradiation, the remainder giving only chromatid changes, was tested and not verified. However, the possibility that the spores differ in the capacity of their nuclei to respond to ultraviolet irradiation, some being at a stage at which no mutation can occur, is not excluded. The high proportion of multiple mutations is in accordance with such a hypothesis. Some of the results of Stadler are interesting in this connection (Stadler, 1939). He found that ultraviolet-irradiated pollen produced deficiencies in the endosperm much more frequently than in the embryo, whereas X-rayed maize pollen yielded a high frequency of deficiencies in both endosperm and embryo. Among seeds which gave only five A deficiencies in the F_1 plants, there were more than a hundred complete A deficiencies in the endosperm. Stadler suggests several possible explanations for this phenomenon, of which the third—namely, that

"there is a difference in some secondary effect after fertilization, for the course and rate of early development are very different in endosperm and embryo"—may be considered here. It may be that ultraviolet treatment causes an effect upon the gene of such a nature that if nuclear division follows very rapidly after the treatment the gene is unable to reproduce itself and a full or fractional deficiency results. However, if division is delayed there may be a possibility of recovery in many cases, so that the number of mutations obtained is greatly decreased. It may be that the effects on the *Neurospora* spores resemble the embryo in that there is a greater chance of recovery than in the endosperm. In order to explain the *Neurospora* ultraviolet data on this basis, we would assume that the irreversible changes would occur more readily in spores at a certain stage. There is some evidence in the work of Swanson (1942) that the response to ultraviolet irradiation is affected by the stage of the nucleus. He treated nuclei in the pollen tubes of *Tradescantia* at various times after sowing on an agar plate and found the number of chromatid breaks to be decreased when the dose was applied at a later than an earlier stage.

An alternative explanation is based on the dual action of the ultraviolet irradiation rather than on heterogeneity among the spores. On this assumption the ultraviolet is believed to have a direct effect on the cytoplasm, apart from its effect in inducing genetic mutations. This effect on the cytoplasm increases with dosage in a cumulative fashion. An interaction takes place between the nucleus and cytoplasm such that mutant nuclei have a smaller chance of surviving in a defective cytoplasm than do normal nuclei. The differential survival value of the normal and mutant nuclei is increased with increased dosage, and this results in a final drop in the mutation rate. In support of this, we have the occurrence of degenerate phenotypes, which show no evidence of being the result of genetic changes but appear rather to be due to cytoplasmic effects. In the case of *Trichophyton,* Hollaender and Emmons (1941) were enabled by suitable treatment of spores after irradiation to increase the mutation rate at higher dosages. This might be due to recovery of the cytoplasm, leading to increased survival of mutated nuclei. The supposition that mutants have an increasingly lower survival rate than normal phenotypes, however, is not in accord with the high frequency of multiple mutations. To account for this, one would have to assume that different mutations differ in respect to their effect on survival, and that deleterious mutations are more frequent at higher dosages. This might be accounted for if the reaction between mutants and cytoplasm is specific; that is, if certain mutants can only survive when certain specific elements in the cytoplasm are uninjured.

COMPARISON OF X-RAY AND ULTRAVIOLET RESULTS.—Because there is no direct method of comparing X-ray and ultraviolet dosage, comparisons between them must be based on biological effects. One such effect is the killing rate of the treatment. The highest mutation rate recorded in the ultraviolet-treated material was 13 per cent, with about 9 per cent survival, whereas with X-ray treatment such a mutation rate would be associated with about 70 per cent survival. There is no doubt, therefore, that X-rays are more effective than ultraviolet irradiation in inducing mutations, when considered in relation to their killing effect. This is in line with the results of Lindegren and Lindegren. They found that less than 1 per cent of the ultraviolet-treated spores survived, and that, of the survivors, about 9 per cent were mutants; whereas in the case of X-rays, about 50 per cent of the treated spores survived, and of these about 25 per cent were mutants. Moreover, it was found that, with ultraviolet irradiation, increase in dosage led to a decrease in the mutation rate, associated with a rapid increase in the killing rate. In the case of X-ray treatment, the number of mutants increased with dosage even up to a dosage of 126,000 r, when only 0.01 per cent of the spores survived the treatment but 78.5 per cent of these were mutants.

The X-ray mutants differed from the ultraviolet mutants in that they were often associated with sterility, whereas ultraviolet mutants were generally fertile. If sterility is usually due to chromosomal aberration, as seems probable, then its frequent occurrence in the X-ray-treated material and its rare occurrence in the ultraviolet-treated material is in accordance with the results of Stadler and his colaborators in maize, and also with the work on *Drosophila*—especially that of Mackenzie and Muller (1940), Demerec, Houlahan, and Hollaender (1942), and Slizynski (1942). The X-ray results indicate that there are at least two types of change leading to the production of visible mutants. The intensity effect seems to indicate that some mutants are directly due to aberrations; whereas the fact that the percentage of mutants among the steriles is not constant, but increases with dosage, indicates that there are some mutants not directly due to aberrations. The data do not enable us to distinguish between mutants that are independent of aberrations ("gene mutations") and mutants that do not result from aberrations but may be associated with them (potential breaks). The rate of occurrence of mutations not resulting from aberrations might afford a more valid basis for comparison with the ultraviolet results than the total mutation rate. Unfortunately, the fact that all aberrations cannot be detected by the present methods makes such a comparison impossible. There is no doubt that the rate of occurrence of such mutations would increase more slowly with dosage than the total mutation rate.

A special type of change, called "degenerate phenotype" by Lindegren and Lindegren, merits further consideration. Degenerate phenotypes either die out or revert to normal on sub-culture, and when crossed with wild-type they give only fluffy and wild-

type progeny. They are therefore thought to be due to an effect on the cytoplasm rather than to a genetic effect. Their frequency is much higher following ultraviolet than following X-ray treatment: the Lindegrens found twelve such types among 36 ultraviolet-induced variants, and one out of 20 among X-ray-induced variants; and we found 40 out of 119 ultraviolet-induced variants, and three out of 99 X-ray-induced variants of this type. This high frequency among the ultra-violet-induced variants is believed to indicate that ultraviolet radiation has a much greater effect upon the cytoplasm than X-radiation, and the high death rate of the ultraviolet-treated spores may be due primarily to the effect of the treatment on the cytoplasm rather than on the nucleus. Any discussion of the possible mode of action of ultraviolet and X-radiation must still be of a highly speculative character. Because of the high absorption coefficient of nucleic acid in the ultraviolet, especially at 2650, it seems probable that the mutational changes produced by ultraviolet are largely caused by initial effects on the nucleic acid. Thus, as was pointed out by Mackenzie and Muller (1940), the ultraviolet radiation is selective in its action on the chromosomes. In contrast to this, the X-rays are not absorbed differentially, but they seem able to cause chromosome breakage wherever a sufficiently large cluster of ionizations occurs (Lea and Catcheside, 1942). It has been shown by Swanson (1942) that ultraviolet radiation will produce chromatid but not chromosome breaks in the pollen-tube chromosomes of *Tradescantia*. What relationship such breaks bear to the visible mutants produced by ultraviolet in *Neurospora* is not known. The characteristic mutation curve shown by ultraviolet-treated spores is most simply explained by heterogeneity of some sort. This is confirmed by the high degree of occurrence of multiple mutants. The simplest hypothesis to explain this would be that not all the microconidia are uninucleate, a certain proportion of them being binucleate. This explanation, however, seems to be precluded by the X-ray results, where there is no evidence of any such heterogeneity. If the heterogeneity resides in the nuclear condition, then certain nuclear stages must be less susceptible to ultraviolet treatment than others, as suggested earlier. If there are certain spores with nuclei that are more responsive to the action of ultraviolet radiation than those of other spores, the question arises as to what happens to these spores when they are subjected to X-radiation. It may well be that such spores give a higher proportion of changes of the more localized type assumed to result from ultraviolet treatment, and if we could distinguish these changes from the other types induced by X-radiation we might obtain a curve for such mutants similar to the ultraviolet dosage-mutation curve.

SUMMARY

The wavelength most effective in inducing mutations in *Neurospora* was 2650. At 2280, the killing rate was high and the mutation rates were low, whereas at 2967 both killing and mutation rates were low unless very high dosages were given.

At 2650, the mutation rate increased with dosage up to a maximum and then decreased as in the case of *Trichophyton*.

Genetic analysis showed 24 out of 60 mutants to be multiple mutants. The rate dosage curve and the high coincidence of mutations are believed to indicate heterogeneity in the treatment given to the spores and in the response of the spore to treatment.

The hypothesis that spores differ in their capacity to give a chromosome rather than a chromatid response to ultraviolet radiation was found insufficient to explain the data, although evidence that both chromatid and chromosome effects occur was obtained.

The occurrence of sterility in association with the mutants was much less frequent in the case of the ultraviolet-induced mutants than in the X-ray-induced mutants.

The ultraviolet results are discussed in relation to the X-ray results and it is suggested that the ultraviolet effects are localized, whereas the X-ray effects are more diversified, including chromosome breaks and rearrangements. The X-ray effects may include effects of the type induced by ultraviolet treatment but this cannot as yet be determined since it is not possible to distinguish between the different types of mutants.

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LITERATURE CITED

COLE, P. A. 1941. The ultraviolet absorption spectra of different regions of *Trichophyton mentagrophytes* spores. Amer. Jour. Bot. 28: 931–934.

DEMEREC, M., A. HOLLAENDER, AND M. B. HOULAHAN. 1942. Effect of monochromatic ultra-violet radiation on *Drosophila melanogaster*. Genetics 27: 139–140.

EMMONS, C. W., AND A. HOLLAENDER. 1939. The action of ultraviolet radiation on dermatophytes. II. Mutations induced in cultures of dermatophytes by exposure of spores to monochromatic ultraviolet radiation. Amer. Jour. Bot. 26: 467–475.

HOLLAENDER, A., AND W. D. CLAUS. 1936. The bactericidal effect of ultraviolet radiation on *Escherichia coli* in liquid suspensions. Jour. Gen. Physiol. 19: 753–765.

———, AND C. W. EMMONS. 1939. The action of ultraviolet radiation on dermatophytes. I. The fungicidal effect of monochromatic ultraviolet radiation on the spores of *Trichophyton mentagrophytes*. Jour. Cell. and Comp. Physiol. 13: 391–402.

———, AND C. W. EMMONS. 1941. Wavelength dependence of mutation production in the ultraviolet with special emphasis on fungi. Cold Spring Harbor Symposia on Quant. Biol. 9: 179–186.

———, K. B. RAPER, AND R. D. COGHILL. 1945. The production and characterization of ultraviolet induced mutations in *Aspergillus terreus*. I. Production of the mutations. Amer. Jour. Bot. 32: 160–165.

———, AND E. M. ZIMMER. 1945. The effect of ultraviolet radiation and X-rays on mutation production in *Pencillium notatum*. (Abstract) Genetics 30: 8.

LEA, D. E., AND D. G. CATCHESIDE. 1942. The mechanism of the induction radiation of chromosome aberrations in *Tradescantia*. Jour. Genetics 44: 216–245.

LINDEGREN, C. C., AND G. LINDEGREN. 1941. X-ray and ultraviolet induced mutations in *Neurospora*. I. X-ray mutations. Jour. Heredity 32: 405–412.

———, AND ———. 1941. X-ray and ultraviolet induced mutations in *Neurospora*. II. Ultraviolet mutations. Jour. Heredity 32: 435–440.

MACKENZIE, K., AND H. J. MULLER. 1940. Mutation effects of ultraviolet light in *Drosophila*. Proc. Roy. Soc. London B 129: 491–517.

SANSOME, E. R., M. DEMEREC, AND A. HOLLAENDER. 1945. Quantitative irradiation experiments with *Neurospora crassa*. I. X-radiation. Amer. Jour. Bot. 32: 218–226.

SLIZYNSKI, B. M. 1942. Deficiency effect of ultraviolet light on *Drosophila melanogaster*. Proc. Roy. Soc. Edin. 61 B: 297–315.

STADLER, L. J. 1939. Genetic studies with ultraviolet radiation. Proc. 7th International Genetical Congress: 269–275.

———, AND G. F. SPRAGUE. 1936. Genetic effects of ultraviolet radiation in maize. I. Unfiltered radiation. II. Filtered radiation. III. Effects of nearly monochromatic 2537 and comparison of effects of X-ray and ultraviolet treatment. Proc. Nat. Acad. Sci. 22: 572–591.

———, AND F. M. UBER. 1942. Genetic effects of ultraviolet radiation in maize. IV. Comparison of monochromatic radiations. Genetics 27: 84–118.

SWANSON, C. P. 1942. The effects of ultraviolet and X-ray treatment on the pollen tube chromosomes of *Tradescantia*. Genetics 27: 491–503.